REMARKS/ARGUMENTS

Claims 1-2, 4-16, 18-30, 57-58 are pending. Claims 2, 17, and 31-56 are cancelled. Claims 57 and 58 are new.

Claims 1 and 2 have been amended to limit the host cell to "uni- or multicellular fungal" host cells. Support for the amendment can be found in paragraph [0115] which states "A lower eukaryotic host cell, when used herein in connection with glycosylation profiles, refers to any eukaryotic cell which ordinarily produces high mannose containing N-glycans, and thus is meant to include some animal or plant cells and most typical lower eukaryotic cells, including uni- and multicellular fungal and algal cells" and in the examples, which teaches construction of recombinant *Pichia pastoris* and *K. lactis* for producing N-glycans comprising $Man_5GlcNAc_2$ or $GlcNAcMan_5GlcNAc_2$.

Claims 1 and 2 were also amended to further limit the host cells to host cells that are diminished or depleted in the activity of an initiating α-1,6-mannosyltransferase. Support for this amendment can be found in paragraph [0174] which states "In a preferred embodiment, the method of the invention involves making or using a host cell which is diminished or depleted in the activity of an initiating α-1,6-mannosyltransferase, i.e., an initiation specific enzyme that initiates outer chain mannosylation on the α-1,3 arm of the $Man_3GlcNAc_2$ core structure. In *S.cerevisiae*, this enzyme is encoded by the *OCH1* gene."

New Claims 57 and 58 depend from Claims 1 and 2, respectively. New Claims 57 and 58 include the subject matter of previously cancelled Claim 3. The subject matter of Claim 3, which recited that "the one or more desired N-glycan structures selected from the group consisting of $Man_3GlcNAc_2$, $GlcNAcMan_3GlcNAc_2$ and $Man_4GlcNAc_2$" and which had been introduced into Claims 1 and 2 in the previous amendment, has been cancelled from Claims 1 and 2.

The Applicant makes the above amendments without prejudice and reserves the right to submit claims that embrace the cancelled subject matter in a subsequent application.

I. Claims 1-2, 4-16, and 18-30 stand rejected under 35 U.S.C. § 112, first paragraph.

Claims 1-2, 4-16, and 18-30 stand rejected under 35 U.S.C. § 112, first paragraph, for allegedly lacking enablement for the method using any non-human host cell. In particular, the rejection states "that while the claims are enabled for yeast lacking och1 and other genes encoding mannosyltransferases, does not provide enablement for any non-human host cell, and

specifically not for a yeast cell with no inactivation of a mannosyltransferase, yet comprising a mannosidase capable of hydrolyzing manα1,3 or Manα1,6 linkages, or both."

Claims 1 and 2 have been amended in the instant amendment to limit the host cell to "a uni- or multicellular fungal host cell which is diminished or depleted in the activity of an initiating α-1,6-mannosyltransferase and which produces N-glycans comprising $Man_5GlcNAc_2$ or $GlcNAcMan_5GlcNAc_2$ structures". The currently amended claims are believed to be enabled for the following reasons.

The specification exemplifies the currently claimed method using unicellular fungal cells such as *Pichia pastoris*, which have been genetically engineered to produce N-glycans comprising $Man_5GlcNAc_2$ or $GlcNAcMan_5GlcNAc_2$ structures. As taught in the specification, *Pichia pastoris* host cells have been genetically engineered to lack the 1,6 mannosyltransferase activity encoded by the host's *OCH1* gene and to further include a nucleic acid that encodes an α-1,2,-mannosidase activity and a nucleic acid encoding a GnT I activity. The above host cells produce N-glycans comprising $Man_5GlcNAc_2$ or $GlcNAcMan_5GlcNAc_2$ structures and can be the starting host cells for Claims 1 and 2.

The applicant further teaches in Example 9, a *K. lactis* strain (another unicellular fungal cell) that has been genetically engineered to lack 1,6 mannosyltransferase activity encoded by the *OCH1* gene and 1,3 mannosyltransferase activity encoded by the *MNN1* gene. Note that *Pichia pastoris*, unlike *K. lactis* and *S. cerevisiae*, lacks an MNN1 gene homolog. Next, a nucleic acid encoding α-1,2,-mannosidase activity and a nucleic acid encoding GnT I activity can be introduced into the genetically engineered *K. lactis* strain following the procedure taught using *Pichia pastoris* to make a *K. lactis* strain that produces N-glycans comprising $Man_5GlcNAc_2$ or $GlcNAcMan_5GlcNAc_2$ structures.

Thus, the Applicant teaches how to construct the starting host cells in several species that have different genetic backgrounds. That is, to practice the applicant's invention, a person of ordinary skill in the art, in light of the instant specification, will either genetically engineer uni- or multicellular fungal host cells to lack 1,6 and/or 1,3 mannosyltransferase activity or isolate a mutant uni- or multicellular fungal host cell that lacks 1,6 and/or 1,3 mannosyltransferase activity and introduce into the host cell an α-1,2,-mannosidase and a GnT I to provide a host cell that produces N-glycans comprising $Man_5GlcNAc_2$ or $GlcNAcMan_5GlcNAc_2$ structures as set forth in the claims. The applicants then teach introducing into the host cell a nucleic acid encoding a mannosidase enzyme that is capable of hydrolyzing *in vivo* an oligosaccharide substrate comprising either or both a Manα1,3 and Manα1,6 glycosidic linkage to the extent that at least 10% of the Manα1,3 and/or Manα1,6

linkages of the substrate are hydrolyzed *in vivo*. Expression of the mannosidase produces one or more desired N-glycan structures on a recombinant glycoprotein expressed in said host cell. In general, the desired N-glycan comprises an oligosaccharide structure selected from the group consisting of $Man_3GlcNAc_2$, $GlcNAcMan_3GlcNAc_2$, and $Man_4GlcNAc_2$. The oligosaccharides can serve as a substrate for additional glycosylation enzymes.

In view of the foregoing amendments and remarks, it is believed that the currently amended Claims satisfy the requirements of 35 U.S.C. § 112, first paragraph, and are in proper condition for allowance. Accordingly, the applicant respectfully requests reconsideration of the rejection and that a Notice of Allowance be forwarded to the applicant. The Examiner is invited to contact applicant's attorney at the telephone number given below, if such would expedite the allowance of this application.

CONDITIONAL PETITION

Applicant hereby makes a Conditional Petition for any relief available to correct any defect in connection with this filing, or any defect remaining in this application after this filing. The Commissioner is authorized to charge deposit account 13-2755 for the petition fee and any other fee(s) required to effect this Conditional Petition.

Respectfully submitted,

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